CRISPR-Cas: To Take Up DNA or Not—That Is the Question

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CRISPR-Cas: To take up DNA or not, that is the question

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Abstract

Historically landmark experiments showed that capsule switching is critical for *Streptococcus pneumonia* survival. Further studies demonstrated that capsule 'transformation' occurs via DNA uptake. In this issue of *Cell Host and Microbe*, Bikard *et al.* (2012) show that CRISPR-Cas systems inhibit DNA uptake, selecting for the outgrowth of CRISPR-defective pneumococci.

To take up DNA or not to take up DNA – that is the question. Each microbe must balance the need to acquire new beneficial traits by horizontal gene transfer (HGT) with the need to prevent the entry of genetic elements that impose fitness costs (Levin, 2010). One system allowing microbes to limit the entry of costly genetic elements is the CRISPR-Cas adaptive immune system found in many bacteria and archaea (Wiedenheft et al., 2012). CRISPR-Cas systems have been shown to inhibit DNA uptake by phage infection, plasmid conjugation, and artificial transformation (Barrangou et al., 2007; Marraffini and Sontheimer, 2008; Wiedenheft et al., 2012). For the 90% of archaea and 50% of bacteria that maintain CRISPR-Cas systems (Wiedenheft et al., 2012), there are apparently more benefits to inhibiting DNA uptake than to promoting it.

Named because they are recognized in microbial genomes as cassettes of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) genes, CRISPR-Cas systems have been the subject of a number of recent studies (Wiedenheft et al., 2012). These investigations have shown that when a microbial genome containing CRISPR- Cas survives an initial phage or plasmid infection, it can acquire a short DNA fragment, known as a 'spacer,' from the invading virus or plasmid. Once transcribed and processed, this CRISPR spacer is used to guide a complex of Cas proteins to destroy invading genetic elements that contain matching DNA sequences (Wiedenheft et al., 2012).

The decision of whether or not to maintain a CRISPR-Cas system depends on a balance of factors that are just beginning to be understood and quantified. *Enterococcus faecalis* represents an interesting example of how this bacterial calculus has been affected by the antibiotic era. *E. faecalis* is a highly evolved inhabitant of the gastrointestinal (GI) tracts of animals, from insects to man. In the complex milieu of the GI tract, there is a high probability of encountering enterococcal phages and potentially other parasitic elements, making CRISPR-mediated defense highly beneficial. However, CRISPR-Cas systems also constitute an impediment to the entry of antibiotic resistance genes conveyed by mobile elements, a barrier that is increasingly costly in the antibiotic era. As a result, a recent

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genomic study found that antibiotic resistance is concentrated in *E. faecalis* strains that have lost CRISPR-Cas defense (Palmer and Gilmore, 2010). This loss of CRISPR-Cas has likely had a profound effect on hospital-adapted *E. faecalis* strains, which now have genomes about 25% larger than those of commensal strains, and are replete with plasmids, phages, pathogenicity islands and other elements (Palmer et al., 2012). Undoubtedly this rapid evolution of hospital isolates has been accompanied by fitness costs in a competitive environment, but in a hospitalized patient treated with antibiotics, colonization by resistant *E. faecalis* is largely non-competitive.

As Bikard et al., (2012) note, *S. pneumoniae* naturally lack CRISPR-Cas loci, whereas other closely related, naturally transformable oral streptococci possess CRISPR-Cas. Given the historic and landmark demonstrations by Griffith (1928) and Avery, MacLeod and McCarty (1944) showing the importance of transformation for immune escape by *S. pneumoniae*, it was of interest to determine whether selection for escape mutants is sufficient to explain the absence of CRISPR-Cas in *S. pneumoniae*. Since no known *S. pneumoniae* strains contain CRISPR-Cas systems, Bikard *et al.,* (2012) grafted a functional CRISPR locus into *S. pneumoniae* from the related species, *S. pyogenes*. They also engineered a *S. pneumoniae* DNA donor to contain a sequence targeted by a *S. pyogenes* CRISPR spacer. This CRISPR-Cas target sequence was placed adjacent to a selectable antibiotic resistance marker. Showing that CRISPR-Cas blocks transformation *in vitro,* Bikard et al., (2012) found that the transplanted CRISPR-Cas system prevents uptake of the resistance marker by *S. pneumoniae*, but only when the donor harbors the adjacent perfect match to the CRISPR spacer. Using very high levels of donor DNA, the investigators were able to obtain a few antibiotic resistant transformants. Yet, these resistant transformants had loss of function mutations in the CRISPR-Cas system or in the target sequence.

Having quantified the effect of CRISPR-Cas as an obstacle to transformation *in vitro*, it was then of interest to determine whether CRISPR-Cas would block the capsule switching observed by Griffith (1928) *in vivo*. Thus, Bikard *et al.,* (2012) inserted a new spacer element identical in sequence to a portion of a *S. pneumoniae* capsule gene into the grafted CRISPR array. The experiments of Griffith (1928) were then repeated: mice were infected with a mixture of heat-killed encapsulated *S. pneumoniae* and live, avirulent un-encapsulated, *S. pneumoniae*. Differing from Griffth's setup, the avirulent mice were now CRISPR+ and loaded with a spacer from a capsule gene. Unlike Griffith's experiments, the mice survived, showing that CRISPR-Cas represents a barrier to capsule switching. However, using higher levels of CRISPR-positive recipients and dead encapsulated donors, a mouse was killed. As in the *in vitro* experiments, the escape mutant that acquired the capsular genes and killed the mouse lacked a functional CRISPR-Cas system. This implies that selection during the course of *S. pneumoniae* infection is strong enough to spur the outgrowth of CRISPR-Cas defective mutants and may explain why *S. pneumoniae* natively lack what appears to be a highly useful system to keep foreign DNA out.

For hospital adapted *E. faecalis* and *S. pneumoniae*, the need for genomic plasticity may mean that the benefits of facile DNA uptake offset the fitness costs associated with lacking CRISPR-Cas' defenses. More generally, one can hypothesize that CRISPR-Cas' frequency in a species is shaped by the probability that a species encounters deleterious foreign DNA. When incoming DNA is mainly harmful to the cell (e.g., lytic viruses), maintaining CRISPR-Cas offers a large selective advantage. Conversely, when microbes require foreign DNA to survive (e.g., under antibiotic targeting), CRISPR-Cas' inhibition of horizontal gene transfer is deleterious. Of course, this tug-of-war selecting for and against CRISPR-Cas may be oversimplified. Some microbes might maintain CRISPR-Cas loci even when HGT is beneficial. As in *E. coli* (Westra et al., 2010), organisms may do so by repressing their CRISPR-Cas systems using genetic switches. When HGT suddenly becomes harmful, these

silent loci could then be switched on to reap CRISPR-Cas' benefits. A whole circuitry for modulating CRISPR-Cas immunity may be ripe for discovery.

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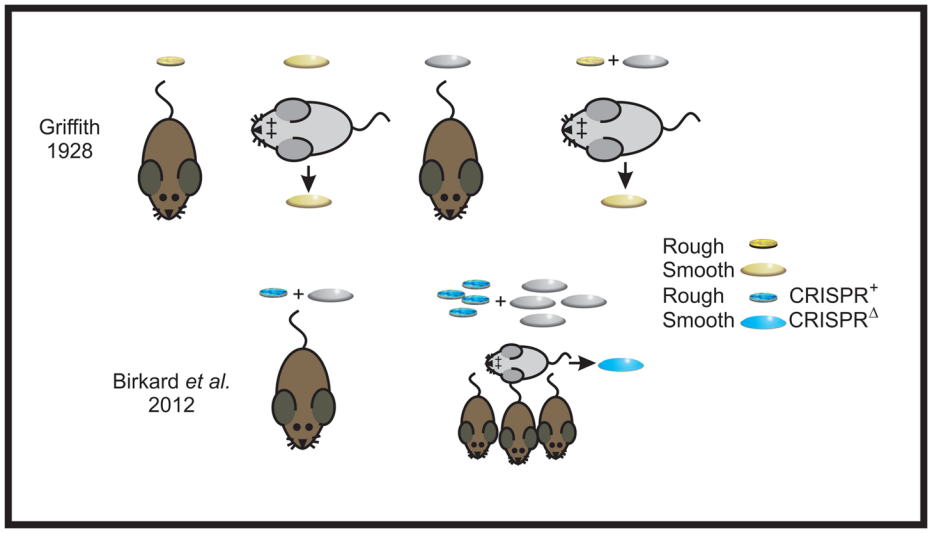

Figure 1.

Top row: The seminal 1928 experiments of Griffith (1928), who found that injection of a rough (un-encapsulated) strain of S. pneumoniae rarely killed mice (top left), whereas injection of a smooth (encapsulated) strain did, and the smooth strain could be recovered from the dead mouse. Injection of heat-killed encapsulated bacteria (grey smooth) did not kill mice, but the combination of live rough *S. pneumoniae* together with heat-killed encapsulated *S. pneumoniae* established a productive infection that killed the mice. Moreover, when the bacteria recovered from the dead mice were cultured, they had 'transformed' from rough to smooth (Griffith, 1928). In the present work (Bikard et al., 2012), Birkard and colleagues showed that if the rough strain carried a functional CRISPR-Cas system that was designed to target a capsule gene, the transformation to smooth, virulent *S. pneumoniae* was blocked. Further, these investigators showed that when they forced the experiment using larger doses of bacteria, one mouse died and smooth bacteria were in fact recovered. These bacteria had mutated their CRISPR-Cas system, rendering it non-functional and unable to block the uptake of capsular DNA.